Evaluation of antibacterial effects of pulp capping agents with direct contact test method

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ABSTRACT

Objectives: Calcium hydroxide has been used in dentistry as a major capping material having the capacity to introduce the formation of a mineralized dentin bridge, but it has no direct inducing effect to the pulp cells. The purpose of this study was to evaluate the antibacterial properties of three different pulp capping agents using a direct contact test (DCT). **Materials and Methods:** The antibacterial properties of three pulp capping agents were evaluated a DCT. For the DCT, wells (n = 12) of 96-microtiter plates were coated with the tested cements (Dycal, Dentsply, USA; DiaRoot BioAggregate, Diadent, Holland; Calcimol LC, Voco, Germany) and Kalzinol (zinc oxide/eugenol cement, Dentsply, USA) was used as control material. A *Lactobacillus casei* suspension was placed on the surface of each specimen for 1 h at 37°C. Bacterial growth was monitored for 16 h with a temperature-controlled microplate spectrophotometer. The kinetics of the outgrowth in each well were recorded continuously at 650 nm every 30 min. The data were analyzed by one-way ANOVA, and Tamhane's T2 multiple comparison test. The level of significance was determined as $P < 0.05$. **Results:** All pulp capping agents showed an increase in the logarithmic growth rate of *L. casei* when compared with the control group ($P < 0.05$). Therefore, all pulp capping agents did not show antibacterial activity. **Conclusions:** The tested pulp capping agents haven't got antibacterial properties. Therefore, they should be used carefully when pulp is exposed or only very thin dentin remained over the pulp to avoid bacterial contamination.

Key words: Antibacterial effects, bacteria, direct contact test, pulp capping agents

INTRODUCTION

Calcium hydroxide ($Ca(OH)_2$) has been used in dentistry as a major capping material having the capacity to introduce the formation of a mineralized dentin bridge, but it has no direct inducing effect to the pulp cells.[1]

In the treatment of carious teeth, infected dentine should always be completely removed. However, infected dentine is sometimes left in the cavity for many reasons. Bacteria left in the cavity are one of the factors leading to secondary caries or pulpal injury after restoration. Antibacterial treatment of the cavity is thus recommended before restoration is completed. Moreover, if the removal of all the dentine affected by caries results in the exposure of tooth pulp in deep lesions, we dentists often try to leave some carious dentine to save the teeth and pulp as much as possible.[2]

A pulp-capping material ought to: (1) Secure the pulp against thermal shocks; (2) disconnect opposed to galvanic action inherent to all amalgam restorations; (3) damage the penetration of mercury from the amalgam restorations into underlying dentin, thus preventing color changes of the tooth; (4) wield an anodyne effect on the pulp; (5) have some antibacterial activity so as to sterilize underlying dentin and residual caries in deep caries lesions; and (6) reduce marginal

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infiltration around restorations, thus restricting the diffusion of bacterial toxins and soluble molecules into underlying dentin and pulp.[3] In order to protect the pulp from secondary infection caused by residual bacteria or microleakage, an ideal pulp-capping agent should have some antibacterial capability.[4]

Products which contain $Ca(OH)_2$ are currently broadly used thanks to their proven properties of stimulating mineralization,[5] protecting the pulp against thermoelectric stimuli, and supporting antimicrobial action. The induction of mineralization appears to be the outcome of extremely the alkaline pH of $Ca(OH)_2$ and its antimicrobial activity is because of the hydroxide ions developing enzymatic inhibition of microorganisms.[6] Nonetheless, the calcium ions have a vital duty in mineralization, thanks to their promotion of cellular migration and differentiation.[7,8]

Due to the stimulation of mineralization, $Ca(OH)_2$ is preferred to use in very deep cavities, especially the situations, which micro exposures could not be clinically noticed. As pulp-capping agents, $Ca(OH)_2$ materials have replaced with zinc phosphate, polycarboxylate, zinc/eugenol cements and with practical advantages especially in terms of their biologic results.[3] Besides their biologic action, calcium and hydroxide ions utilize an antimicrobial action with the former reacting carbonic gas in order to remove the source of respiration of anaerobic bacteria and the latter preventing the bacterial enzymatic system.[5,6]

Direct pulp capping is estimated an efficient treatment method in today's endodontics, because successful capping can preserve tooth vitality in an exposed pulp cavity. Under normal conditions, $Ca(OH)_2$ preparations are used as capping materials. The main advantage of $Ca(OH)_2$ is its biological activity. It presents antimicrobial and anti-inflammatory activities principally due to the high pH value of the surrounding environment (around 12.5) following its dissolution. Most bacteria can not oppose a pH above 9.5 and the alkalinity allows the resolution of the exudates, which maintain the inflammatory state. $Ca(OH)_2$ works as a chemical buffer because of this alkalinity and as a thermal buffer against metallic materials because of its low-thermal conductivity.[1]

In various clinical conditions, it is possible to accidentally reveal the dental pulp during tooth preparation and the extraction of decayed dentin. Direct pulp capping might be showed in selected cases for maintaining pulp vitality and function. So, the capping material could be a key factor in deciding the treatment result. An ideal direct pulp capping material ought to control infection, maintain dentin tightly (to prevent microleakage), be clinically simple to handle and finally, improve dentin bridge development.[9]

The purpose of this study was to evaluate the antibacterial properties of three different pulp capping agents using a direct contact test (DCT).

MATERIALS AND METHODS

Pulp capping agents and control material used in this study are shown in Table 1.

Lactobacillus casei (Refik Saydam National Public Health, 900) was grown aerobically from frozen stock cultures in brain hearth infusion (BHI) broth containing 0.5% bacitracin for 48 h at 37°C before applying it to the specimens according to the experimental design.

DCT

The DCT[10] is based on the turbidimetric determination of bacterial growth in 96-well microliter plates (96-well, flat-bottom Nunclon, Nunc, Copenhagen, Denmark). The kinetics of the outgrowth in each well was recorded continuously at 650 nm every 30 min, using a temperature-controlled spectrophotometer (μquant, Bio-Tek Instruments Inc., Winooski VT, USA). In all the wells, the sidewall was coated with the tested material while the plate was held vertically (i.e., the plate's surface was perpendicular to the floor). Pulp

Table 1: Materials used in this study

Materials	Compositions	Manufacturer
Dycal®	Base paste: 1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, calcium tungstate, iron oxide pigments Catalyst paste: $Ca(OH)_2$, n-ethyl-o/p-toluene sulfonamide Zinc oxide, titanium dioxide, zinc stearate Iron oxide pigments (dentine shade only)	Dentsply Caulk Lakeview and Clark Avenues Milford, DE 19963-0359 USA
Calcimol LC	Methacrylate and amines	VOCO GmbH P.O. Box 767 27457 Cuxhaven Germany
DiaRoot® BioAggregate	Gel-like calcium silicate hydrate $Ca(OH)_2$ (less than MTA) Hydroxyapatite Tantalum oxide Amorphous silicon oxide	Diadent Antennestraat 70, 1322 AS Almere, Netherlands

MTA: Mineral trioxide aggregate, $Ca(OH)_2$: Calcium hydroxide

capping agents mixed and applied to the sidewalls with the manufacturers' recommendations of the wells. Care was taken to have a thin film thickness. In this study, Kalzinol (Zinc-oxide Eugenol cement) was used as a control material. A 10 μL bacterial suspension was placed on each sample and incubated while the plate remained in a vertical position for one hour at 37°C. During that time, most of the suspension liquid evaporated, ensuring direct contact between all bacteria and the tested material surface. Then, 220 μL of BHI broth was added to each of the wells and the plate was placed in the spectrophotometer. The bacterial outgrowth was estimated after direct contact with the tested material on the basis of the changes in the readings of optical density at 650 nm, which were recorded by the spectrophotometer every 30 min for 19 h.

Statistical analyses

Bacterial growth curves for each well were analyzed and a regression line on the ascending linear portion of the curve was calculated, using the equation $y = ax + b$. This equation provided the value of the slope corresponding to the growth rate. The data were analyzed by one-way ANOVA, as well as by Tamhane's T2 multiple comparison test. The level of significance was determined as $P < 0.05$.

RESULTS

The *L. casei* growth in a 96-well microtiter plate is shown in Figure 1. Each point on the growth curve is the average of the optic density measured in eight wells at any given time in Figure 1. Each curve includes 32 measurements taken within 19 h.

The results of the DCT showed that all pulp capping agents showed an increase in the logarithmic growth rate of *L. casei* when compared with the control group ($P < 0.05$). Therefore, all pulp capping agents did not show antibacterial activity.

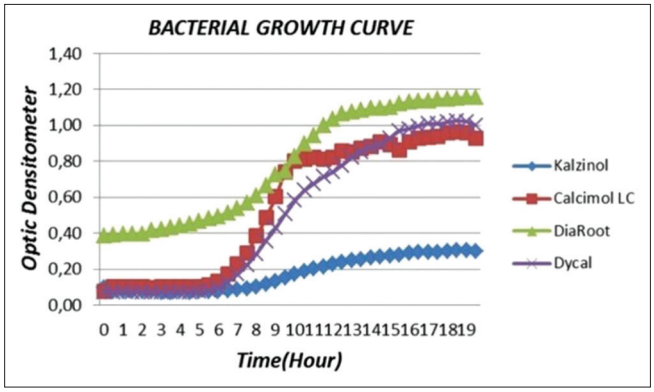

Figure 1: Growth curves of bacterial outgrowth after direct contact between *Lactobacillus casei* and tested materials. Each point on the growth curve represents the average optical density measured at 650 nm in eight wells

DISCUSSION

The antibacterial action of dental substances has been solidly tested with both agar diffusion test (ADT) and DCT.[11-13] The ADT was found more effective in the detecting antibacterial properties of dental materials than DCT.[11] According to calibration growth curves, the DCT permits for assessment of the number of feasible bacteria at the end of the direct contact incubation period with a temperature-controlled spectrophotometer and the appropriate software.

The DCT quantitatively measures the effect of direct and close contact between the test microorganism and the tested materials, regardless of the solubility and diffusibility of their components.[10]

Direct pulp capping of teeth exposed during caries removal has not been viewed as mainstream treatment for either primary or permanent teeth.[14] Stanley and Cox are at the forefront of a movement to disprove the axiom "the exposed pulp is a doomed organ." They wish us to consider pulp as a tissue with many more regenerative powers than has been traditionally held.[15]

According to Stanley,[14] inorganic CH, with its very basic pH, creates zones of obliteration and coagulation necrosis superficial to the deeper zones where reparative dentine recently begins. Thus in the process of initiating repair, CH injures the pulp. The lower pH CH products such as Dycal may get rid of major tissue damage and stimulate reparative dentine more directly.

According to recent studies, the classification and clinical indications of various formulations of $Ca(OH)_2$[16,17] and their mechanisms of antimicrobial activity[18] clearly indicate that this medication has been applied for more than 80 years, even though there are still many questions so as to be answered concerning its antimicrobial action.

$Ca(OH)_2$-based materials are prevalently used as antimicrobial materials in dental practices, e.g. as cavity liners, pulp-capping agents or for the treatment of infected root canals. $Ca(OH)_2$ can be applied either as suspension in an inert solvent (water, glycerin) or self-setting cements may be formed by the reaction

of $Ca(OH)_2$ and salicylate ions to form calcium salicylate (with an unreacted $Ca(OH)_2$ component) or by adding $Ca(OH)_2$ to light-cured methacrylate-based resins.[19]

The antimicrobial and anti-inflammatory potency of $Ca(OH)_2$ cements originates from the release of hydroxyl ions, which raise the pH value of the surrounding environment to approximately 12-12.5 after dissolution. This high pH value may kill bacteria by damaging the cytoplasmic membrane and DNA and denaturing proteins.[18]

$Ca(OH)_2$ cements have some drawbacks in addition to being weak. While suspensions or salicylate-based systems release sufficient $Ca(OH)_2$ for significant antibacterial properties[20] and the stimulation of secondary dentine,[21] they are thought to be soluble.[22] Light-cured systems are less soluble; however, there is little proof of considerable antibacterial effect by them.[20]

$Ca(OH)_2$ is the most qualified applicant for direct pulp capping. Nevertheless, in clinical practice, softening and disintegration of $Ca(OH)_2$ might happen during the acid etching procedure prior to an adhesive restoration.[23] These upshots may lead to contamination of the bonding agents and promote the potential for microleakage. Furthermore, it is considered that the highly alkaline pH of $Ca(OH)_2$ could menace pulp vitality.[24]

Hard-setting $Ca(OH)_2$ cements might cause the formation of dentin bridges. Still, they don't provide an effective long-term seal against bacterial factors. Within a few years, the majority of mechanically exposed and capped pulps indicate infection and necrosis because of microleakage of capping materials and tunnel defects in the dentin bridges. It is unknown that newer types of resin containing calcium-hydroxide-products will perform as a permanent barrier.[25]

$Ca(OH)_2$ also presents some deficiencies: (i) It incites pulp necrosis during the first days, then the pulp reacts by establishing an atubular tertiary dentine bridge, but this dentine formation is made to the detriment of the pulpal volume with long-term biological consequences; (ii) when the paste is only $Ca(OH)_2$, its application in the root canal system is easy but the low hardening and the retraction by drying do not allow tight fillings, consequently it is only used as temporary material in this indication for which hermeticity is a priority; (iii) to get round this disadvantage, i.e. to increase the crushing strength and to decrease the setting time, polymeric bases were added, but under these conditions the setting time is too short to use these materials as root canal filling.[1]

CONCLUSIONS

Within the limitations of this study, the tested pulp capping agents haven't got antibacterial properties. Therefore, they should be used carefully when pulp is exposed or only very thin dentin remained over the pulp to avoid bacterial contamination. Further studies are required to investigate the long-lasting antibacterial properties of the tested materials.

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